CORRECTED DECLARATION

Declarations executed by co-inventors Wise, Kuske and Terwilliger are submitted herewith, correcting the defect noted by the Examiner in Paper No. 4.

AMENDMENTS TO THE CLAIMS

IN THE CLAIMS:

Please cancel claim 8.

1. (Twice Amended) A method for enhancing [a response of] transcriptional activation of a reporter gene under the control of a promoter regulated by a DmpR protein in bacteria selected from the group consisting of *Pseudomonas [putida, Acinetobacter]* and *Escherichia coli* in response to phenols and substituted phenols over the [response] transcriptional activation exhibited by wild type bacteria of the same strain, [said bacteria having a regulatory protein selected from the group consisting of DmpR, MopR, PhhR, PhlR, XylR, and TbuT with discrete functional domains for independent activities including a sensor domain that detects said phenols and substituted phenols through a direct physical interaction forming a protein-molecule complex which binds to a cognate promoter sequence and activates expression of genes encoding metabolic enzymes, a DNA-binding region, and a transcription activation region,] said method comprising the steps of removing the sensor domain from the [bacterial] DNA encoding the [regulatory] DmpR protein, subjecting the removed sensor domain to mutagenic polymerase chain reaction, ligating the mutated sensor domain into the DNA encoding the [regulatory] DmpR protein, and testing the bacteria for enhanced response to said phenols and substituted phenols over the response thereto for wild type bacteria without altering other domains.

9. (New) A method of detecting the presence of 2-chlorophenol in a test sample, comprising

(a) culturing *Pseudomonas or Escherichia coli* bacteria in the presence of the test sample, said bacteria containing a DmpR gene and a reporter gene under the control of a promoter inducible by DmpR, said DmpR gene containing a mutation in the sensor domain conferring an enhanced transcriptional activation response to 2-chlorophenol relative to wild type DmpR, and

(b) detecting the expression of the reported gene,

wherein the expression of the reporter gene provides an indication of the presence of 2-chlorophenol in the test sample.

10. (New) The method according to claim 9, wherein the DmpR mutant is selected from the group consisting of DmpR-B21, DmpR-B23, and DmpR-D9.

11. (New) A method of detecting the presence of 2,4-dichlorophenol in a test sample, comprising

(a) culturing *Pseudomonas or Escherichia coli* bacteria in the presence of the test sample, said bacteria containing a DmpR gene and a reporter gene under the control of a promoter inducible by DmpR, said DmpR gene containing a mutation in the sensor domain conferring an enhanced transcriptional activation response to 2,4-dichlorophenol relative to wild type DmpR, and

(b) detecting the expression of the reported gene,

wherein the expression of the reporter gene provides an indication of the presence of 2,4-dichlorophenol in the test sample.

12. (New) The method according to claim 11, wherein the DmpR mutant is selected from the group consisting of DmpR-B21, DmpR-B17#2, DmpR-B9 and DmpR-D12.

13. (New) A method of detecting the presence of 2,4-dimethylphenol in a test sample, comprising

(a) culturing *Pseudomonas or Escherichia coli* bacteria in the presence of the test sample, said bacteria containing a DmpR gene and a reporter gene under the control of a promoter inducible by DmpR, said DmpR gene containing a mutation in the sensor domain conferring an enhanced transcriptional activation response to 2,4- dimethylphenol relative to wild type DmpR, and

(b) detecting the expression of the reported gene,

wherein the expression of the reporter gene provides an indication of the presence of 2,4-dimethylphenol in the test sample.

14. (New) The method according to claim 13, wherein the DmpR mutant is DmpR-B31.

15. (New) A method of detecting the presence of 2-nitrophenol in a test sample, comprising

(a) culturing *Pseudomonas or Escherichia coli* bacteria in the presence of the test sample, said bacteria containing a DmpR gene and a reporter gene under the control of a promoter inducible by DmpR, said DmpR gene containing a mutation in the sensor domain conferring an enhanced transcriptional activation response to 2-nitrophenol relative to wild type DmpR, and

(b) detecting the expression of the reported gene,

wherein the expression of the reporter gene provides an indication of the presence of 2-nitrophenol in the test sample.

16. (New) The method according to claim 15, wherein the DmpR mutant is DmpR-D9.

17. (New) A method of detecting the presence of 4-nitrophenol in a test sample, comprising

(a) culturing *Pseudomonas or Escherichia coli* bacteria in the presence of the test sample, said bacteria containing a DmpR gene and a reporter gene under the control of a promoter inducible by DmpR, said DmpR gene containing a mutation in the sensor domain conferring an enhanced transcriptional activation response to 4-nitrophenol relative to wild type DmpR, and

(b) detecting the expression of the reported gene,

wherein the expression of the reporter gene provides an indication of the presence of 4-nitrophenol in the test sample.

18. (New) The method according to claim 17, wherein the DmpR mutant is DmpR-B31.

19. (New) A method of detecting the presence of phenol in a test sample, comprising

(a) culturing *Pseudomonas or Escherichia coli* bacteria in the presence of the test sample, said bacteria containing a DmpR gene and a reporter gene under the control of a promoter inducible by DmpR, said DmpR gene containing a

mutation in the sensor domain conferring an enhanced transcriptional activation response to phenol relative to wild type DmpR, and

(b) detecting the expression of the reported gene,

wherein the expression of the reporter gene provides an indication of the presence of phenol in the test sample.

20. (New) The method according to claim 19, wherein the DmpR mutant is DmpR-B9.

21. (New) A method of detecting the presence of one or more phenolic compounds selected from the group consisting of phenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4-dimethylphenol, 2-nitrophenol and 4-nitrophenol in a test sample, comprising

(a) culturing *Pseudomonas or Escherichia coli* bacteria in the presence of the test sample, said bacteria containing a DmpR gene and a reporter gene under the control of a promoter inducible by DmpR, said DmpR gene containing a mutation in the sensor domain conferring an enhanced transcriptional activation response to the phenolic compound(s) relative to wild type DmpR, and

(b) detecting the expression of the reported gene,

wherein the expression of the reporter gene provides an indication of the presence of one or more phenolic compounds in the test sample.

22. (New) An isolated polynucleotide consisting of a nucleotide sequence selected from the group consisting of SEQ ID NOS. 1 - 7, and complementary sequences thereof.

23. (New) A polynucleotide vector comprising the polynucleotide according to claim 22.

24. (New) A host cell containing the vector of claim 23.

25. (New) A method of detecting the presence of a phenolic compound selected from the group consisting of phenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4-dimethylphenol, 2-nitrophenol, 4-nitrophenol and 4-chloro-3-methylphenol in a test sample, comprising

(a) culturing a bacteria in the presence of the test sample, wherein the bacteria is selected from the group consisting of *Pseudomonas* and *Escherichia coli* and contains a reporter gene under the control of a promoter inducible by a mutant DmpR protein having at least a 4-fold enhanced transcriptional activation response to said phenolic compound relative to wild type DmpR, and

(b) detecting the expression of the reported gene,

wherein the expression of the reporter gene provides an indication of the presence of the phenolic compound in the test sample.

REMARKS

Claim 8 has been cancelled. Claim 1 is pending and has been amended. New claims 9 - 25 have been added. No new matter has been introduced by these amendments.

THE INVENTION

The subject invention is directed to the detection of organic pollutants (e.g., phenols and substituted phenols) using bacteria engineered to express sensor-domain mutants of metabolic enzyme regulator proteins such as DmpR. The mutant regulator proteins provided by the invention are able to recognize organic effector molecules that the corresponding wild type regulators do not, or are only able to recognize at very high concentrations. The described methods for detecting an organic pollutant utilize